Characterization of the Properties of Mouse-Adapted Human Measles Virus[1] (34333)

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The Edmonston strain of human measles virus was adapted to growth in the brains of newborn mice by Imagawa and Adams (1). This mouse-adapted measles virus produces a characteristic encephalitis in intracerebrally-inoculated mice after a period of 5–8 days, unlike the parental Edmonston strain of measles virus which causes no observable effects following inoculation. The present study was undertaken to characterize the replication of the mouse-adapted measles virus in tissue culture. The ability of the virus to induce interferon and the effects of actinomycin D on replication of the virus were also investigated.

Materials and Methods. Virus. The mouse-adapted measles virus was originally obtained from Dr. Imagawa and has undergone numerous serial passages in newborn mice in our laboratory. A 10% brain suspension of the virus was made from infected brains harvested when the mice appeared sick. The diluent was 0.5% lactalbumin hydrolysate in Earle's basal salt solution supplemented with 10% fetal bovine serum (FBS), antibiotics (300 units of penicillin and 300 μg of streptomycin/ml), and 0.075% sodium bicarbonate. Prior to this study, the mouse-adapted measles virus had also undergone a number of serial passages in BSC-1 and in Vero cells, both stable lines derived from African green monkey kidneys (2, 3). Virus stocks were prepared by inoculation of BSC-1 or Vero 16-oz bottle cultures with the virus, which was harvested 2–3 days later by disruption of the cells with two cycles of freezing and thawing. Cell debris was removed by low speed centrifugation and the supernatant was dispensed in 2-ml ampoules. The virus was quick-frozen and stored at —65° until used.

Cells. Primary African green monkey kidney cells were grown in 0.5% lactalbumin hydrolysate in Hanks' balanced salt solution supplemented with 2% FBS. The BSC-1 cell line was received from Dr. R. Dulbecco, and the Vero cell line was supplied by Dr. J. Desmyter. The BSC-1 and Vero cells were grown in Eagle's basal medium with 10% FBS, 10% tryptose phosphate broth (TPB), and 0.075% sodium bicarbonate. All media contained 100 units of penicillin and 100 μg of streptomycin/ml. When petri dish cultures were used, the cells were grown in 5% CO_2 with the same medium as described above except that 0.23% sodium bicarbonate was used.

Actinomycin D experiments. Actinomycin D (AD), at a concentration of 0.1 μg/ml in Eagle's medium, was added to one-half of the BSC-1 or Vero cell tube cultures containing 3–5 $\times$ 10^5 cells/tube, 2 hours prior to virus infection. The cells were then exposed to a known multiplicity of the mouse-adapted measles virus, and the virus was allowed to adsorb 90 min at room temperature. The cell sheet was then washed twice with Tris buffer

[1] Supported in part by Public Health Service research grant CA 10036 from the National Cancer Institute, National Institutes of Health.

[2] Predoctoral fellowship No. 5-F1-GM-37,590-02 from the National Institute of General Medical Sciences, National Institutes of Health.

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(pH 7.4) and medium, with or without AD, was added, the medium containing the AD being added to the cultures pretreated with AD. At fixed intervals, two tubes of each sample were harvested by two cycles of freezing and thawing, the cell debris was removed by low speed centrifugation, and the supernatant was titrated.

Viral assay. All virus titrations were carried out using the plaque technique in the various cell lines as previously described by Rapp (4) except that the second overlay was omitted and plaques were stained on the fourth day with 2 ml of a 1:7500 dilution of neutral red.

Induction and assay of interferon. Interferon induced by the mouse-adapted measles was prepared by the technique suggested by Desmyter *et al.* (5) and described in detail by Mirchamsy and Rapp (6). After 72-hr incubation of virus with the cells, the culture fluids were decanted, acidified to pH 2 for 48 hr, adjusted to neutrality and centrifuged two times in a Spinco model L2 ultracentrifuge. To assay the interferon, 3.5 ml of Eagle's plus 2% FBS was added to the petri dish cultures of BSC-1 or Vero cells. Then 0.5-ml dilutions of interferon were added and allowed to incubate for 18 hr at 37° in a CO_2 incubator. The fluids were then removed, the cells were washed with Eagle's medium and challenged with 70–100 plaque-forming units of vesicular stomatitis virus. The challenge virus was allowed to adsorb for 1 hr, and the cultures were then overlaid with a medium consisting of 1% agar in Eagle's medium, 10% FBS, 1:30,000 dilution of neutral red, and 0.23% sodium bicarbonate. The interferon titer was expressed as the reciprocal of the interferon dilution which resulted in a 50% reduction in the number of plaques induced by vesicular stomatitis virus.

Results. Cytopathic effects in cell lines. The mouse-adapted measles virus was grown on coverslips of Vero and BSC-1 cells; and pairs of coverslips infected with virus or uninfected controls were removed everyday, washed twice in saline, fixed with Bouin's fixative and stained with hematoxylin and eosin for observation of the cytopathic effect produced by the virus. The results obtained showed that giant cells developed much faster (within 24 hr) and were larger in the Vero cells than in the BSC-1 cells. Intracytoplasmic inclusions formed in both cell systems, but intranuclear inclusions were absent in Vero cells even 7 days after inoculation of the virus. In BSC-1 infected cells, intranu-

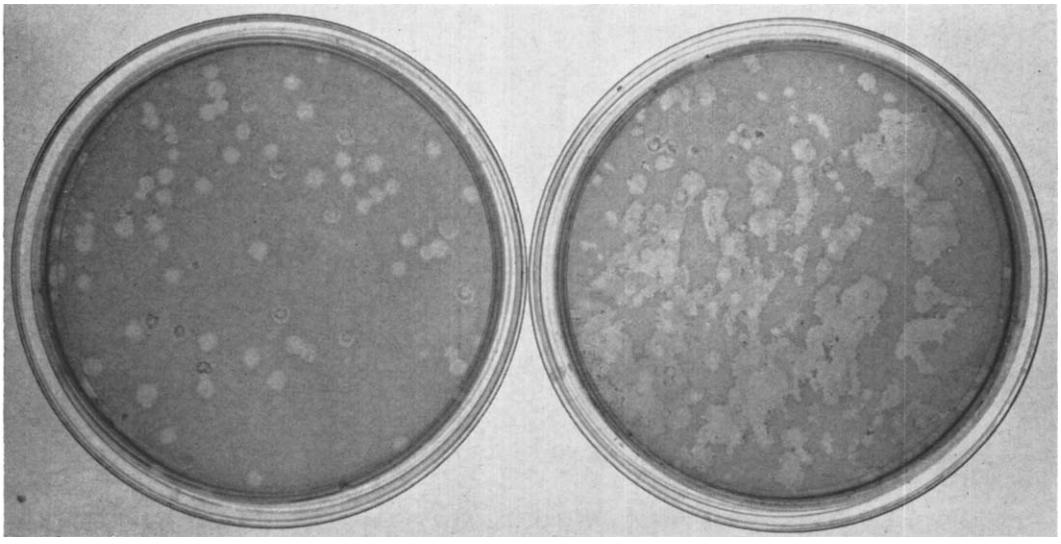

FIG. 1. BSC-1 cells 5 days after inoculation of mouse-adapted measles virus (left) and Edmonston measles virus (right); the cells were maintained under an agar overlay to prevent secondary plaques from developing.

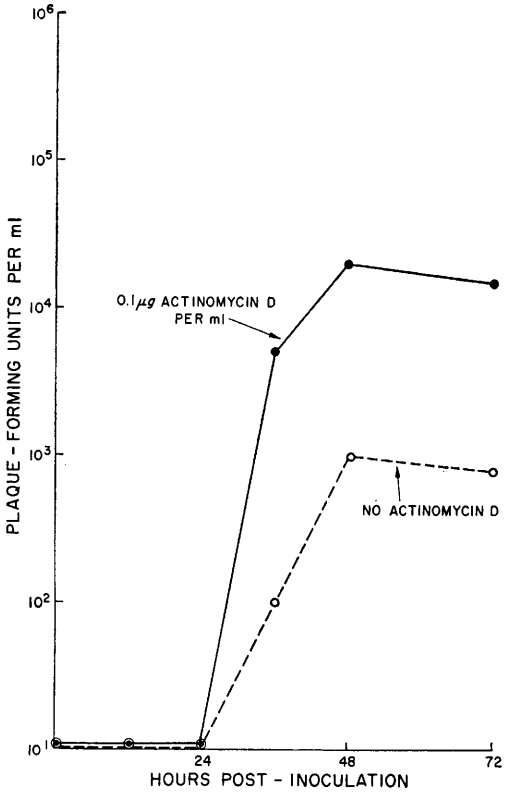

FIG. 2. Replication of mouse-adapted measles virus in BSC-1 cells in the presence and absence of actinomycin D.

clear inclusions were observed at a late stage of infection. When mouse-adapted measles was grown in primary green monkey kidney cells, virus-induced CPE appeared within 24 hr and progressed more rapidly than it did in BSC-1 cells, where giant cells generally appeared between 36 and 48 hr.

Plaque morphology. Differences in plaque morphology in BSC-1 cells induced by the mouse-adapted measles virus or by the Edmonston measles virus are shown in Fig. 1. The mouse-adapted virus produced round plaques of 2–3 mm in diameter within 5 days (Fig. 1, left) which fail to increase markedly in size on prolonged incubation, unlike the plaques formed by the human Edmonston strain which continue to enlarge and to "comet" (Fig. 1, right). The mouse-adapted virus plaques do not "comet" or "run" as do the human measles virus plaques in the BSC-1 cells [Ref. (4) and Fig. 1]. Plaques of the mouse-adapted measles virus in primary green monkey kidney or Vero cells appear similar to those seen in the BSC-1 cell line.

Effect of actinomycin D on the replication of mouse-adapted measles virus. To study the effect of AD on the replication of the mouse-adapted measles in either BSC-1 or Vero cells, the virus was inoculated onto the BSC-1 or Vero cells in tubes at a multiplicity of 0.1–0.4 plaque-forming units of virus/cell. Half of the tube cultures had been pretreated and then maintained in the presence of 0.1 μg/ml of AD. Figure 2 shows the replication of the mouse-adapted measles in BSC-1 cells in the presence and absence of AD. The virus underwent an eclipse period of about 24 hr, and then increased to a titer of about 10^3 plaque-forming units/ml in the absence of actinomycin D within 48 hr after inoculation of the cultures. In the presence of AD, the virus underwent a similar latent period, but subsequently increased in titer to about 2×10^4 plaque-forming units/ ml. This rise was

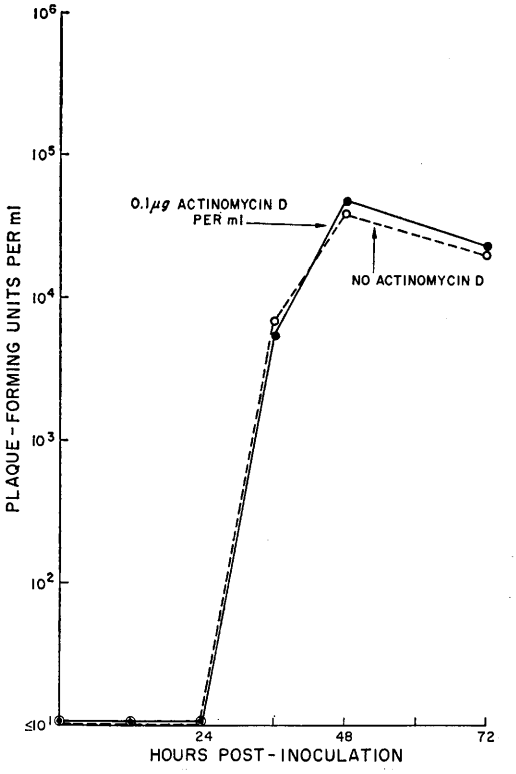

FIG. 3. Replication of mouse-adapted measles virus in Vero cells in the presence and absence of actinomycin D.

more rapid than that observed in the untreated cultures.

A similar experiment was performed to study the replication of mouse-adapted measles in the presence and absence of AD in Vero cells. The results are shown in Fig. 3. Unlike the results in BSC-1 cells, actinomycin D had no effect on the yield of mouse-adapted measles from Vero cells. The virus again exhibited a latent period greater than 24 hr and then increased in both the presence and absence of AD to a maximum titer at 48 hr of about 5×10^4 plaque-forming units/ml. Numerous experiments verified these results.

Interferon induction in BSC-1 and Vero cells. To study interferon induction in BSC-1 and Vero cells by the mouse-adapted measles, 16-oz bottle cultures of Vero and BSC-1 cells were infected with the mouse-adapted measles at a multiplicity of 0.01 PFU/cell. After 72-hr incubation, the culture fluids were treated as described in "Materials and Methods." The supernatant was assayed in both BSC-1 and Vero cells for interferon activity. Table I shows the results of the comparative induction of interferon in BSC-1 and Vero cells by the mouse-adapted measles virus. The adapted measles virus in BSC-1 cells induced a titer of interferon of 16–32 when assayed in BSC-1 cells and 8–16 when assayed in Vero cells. Interferon titers were, therefore, slightly lower when assayed in Vero cells as compared to titers obtained in BSC-1 cells. No detectable level of interferon could be demonstrated as a result of mouse-adapted measles infection of Vero cells although the assays for interferon were carried out in both BSC-1 and in Vero cells.

TABLE I. Induction of Interferon in BSC-1 and Vero Cells by Mouse-Adapted Measles Virus.

Origin of interferon	Titer[a] of interferon in	
	BSC-1	Vero
BSC-1	16–32	8–16
Vero	<8	<8

[a] Reciprocal of interferon dilution capable of reducing the number of plaques formed by vesicular stomatitis virus by 50%.

Discussion. The effect of AD on yields of the RNA-containing arboviruses (7, 8), Newcastle disease virus (9), and human measles virus (6) has been postulated to be the result of inhibition of induction of interferon. This idea correlates with our present data. The yield of the mouse-adapted measles virus is increased by AD treatment of BSC-1 cells, the cell line in which the virus also induces interferon, but the yield of the virus is not increased by AD treatment of virus-infected Vero cells, a cell line in which the virus induces little or no interferon. This supports the findings by Desmyter *et al.* (10) that the Vero cell line is unable to produce interferon following exposure to many different interferon-inducing viruses. Measles virus was first shown to induce the synthesis of interferon by Ho and Enders (11) and DeMaeyer and Enders (12). That attenuation of measles virus was accompanied by ability to induce larger amounts of interferon than that induced by virulent strains was suggested by Enders (13) and DeMaeyer and Enders (14).

It is, therefore, of interest to compare the properties of the mouse-adapted measles virus with other human strains of the virus. Mirchamsy and Rapp (6) demonstrated that AD treatment of BSC-1 cells increased the yield of both the Edmonston virulent strain and the Schwarz attenuated strain of measles virus, but that yields of the Schwarz virus from Vero cells were the same, regardless of whether the cultures had been treated with AD. Studies on the production of interferon by these two viruses showed that the Edmonston virulent strain failed to induce detectable levels of interferon in BSC-1 cells while the Schwarz attenuated strain produced interferon in these cells. The present data suggest that the mouse-adapted measles virus can induce interferon and is, therefore, more similar to the Schwarz attenuated strain of human measles virus than the parental Edmonston strain in this regard. The results also suggest that the increase in titer in BSC-1 cells in the presence of AD is due to interference with the production of interferon by these cells.

Summary. The mouse-adapted measles vi-

rus induced plaques of different morphology from the parental Edmonston measles virus in primary green monkey kidney cells and in stable cell lines (BSC-1 and Vero) derived from this tissue. The CPE produced by the mouse-adapted measles appeared earlier in the Vero and primary green monkey kidney cells than in the BSC-1 cells. Actinomycin D increased the yield of the mouse-adapted measles in BSC-1 cells but not in Vero cells. This correlates well with the production of interferon in BSC-1 cells and lack of production of interferon in Vero cells following inoculation of the cultures with the mouse-adapted measles virus.

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Received June 23, 1969. P.S.E.B.M., 1969, Vol. 132.